PATENT COOPERATION TREATY (Translation made by Sonoda & Kobayashi)

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/JP03/16496	International filing date *(day/month/year)* 22. 12. 2003	Priority date *(day/month/year)* 26. 12. 2002
International Patent Classification (IPC) or national classification and IPC Int. Cl.7 C12N 15/63, C12 N 5/10, C07K 2/00		
Applicant RIKEN		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 3 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☒ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) one floppy disk, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
30. 07. 2004	16. 03. 2005
Name and mailing address of the IPEA/ Facsimile No.	Authorized officer Telephone No.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP/03/16496

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☐ the international application in the language in which it was filed

☐ a translation of the international application into _____ , which is the language of a translation furnished for the purposes of:

☐ international search (Rules 12.3(a) and 23.1(b))

☐ publication of the international application (Rule 12.4(a))

☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

☐ the international application as originally filed/furnished

☒ the description:

pages 1-17 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages 2, 4-8, 10-16 as originally filed/furnished

pages* _____ as amended (together with any statement) under Article 19

pages* 1-9 received by this Authority on 08. 11. 2004

pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-5 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☒ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☒ the claims, Nos. 3

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP03/16496

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 12-16, and among Claims 1, 2, 4-11, those portions not relating to DT40 cells

because:

☐ the said international application, or the said claims Nos. _____ relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _____ are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed *(specify)*:

☒ no international search report has been established for said claims Nos. 12-16, and among Claims 1, 2, 4-11, those portions not relating to DT40 cells

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Preliminary Examining Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13*ter*.1(a) or (b) and 13*ter*.2.

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Preliminary Examining Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP03/16496

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	2, 4-11	YES
	Claims	1	NO
Inventive step (IS)	Claims		YES
	Claims	1, 2, 4-11	NO
Industrial applicability (IA)	Claims	1, 2, 4-11	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Citation 1: NICKOLOFF, J. A., Mol. Cell. Biol., (1992), Vol. 12, No. 12, pp. 5311-5318
Citation 2: BUERSTEDDE, J. and TAKEDA, S., Cell, (1991), Vol. 67, No. 1, pp. 179-188
Citation 4: LAHTI, J. M., Methods, (1999), Vol. 17, No. 4, pp. 305-312
Citation 5: SLEBOS, R. et al., Biochem. Biophys. Res. Commun., (2001), Vol. 281, No. 1, pp. 212-219
(Herebelow are citations newly found at the International Preliminary Examination Stage)
Citation 3: PHI-VAN, L. and Stratling, W. H., Biochemistry, (1996), Vol. 35, No. 33, pp. 10735-10742
Citation 6: BULFONE-PAUS, S. et al., Nucleic Acids Res., (1995), Vol. 23, No. 11, pp. 1997-2005
Citation 7: LAUSTER, R. et al., EMBO J., (1993), Vol. 12, No. 12, pp. 4615-4623
Citation 8: ISRAEL, I. D., Nucleic Acids Res., (1989), Vol. 17, No. 12, pp. 4589-4595

Citation 1 describes a method of inducing homologous recombination in somatic animal cells wherein, during homologous recombination between a gene such as neo whereof the transcription is controlled by a DEX (dexamethasone) inducible MMTV promoter, which is incorporated into a chromosome of a somatic animal cell such as a CHO cell, and a different gene such as neo, the efficiency of homologous recombination is improved by activating transcription from the aforementioned DEX inducible MMTV promoter.

Citation 2 describes that in a system that measures the frequency (targeted/total integration) at which homologous recombination (Targeted Integration) occurs between DNA with a sequence highly homologous to a gene such as the β-actin gene that is transduced into a cell, and a gene such as the β-actin gene on a chromosome in the aforementioned cell, when DT-40 is used as the cell, the frequency (efficiency) of homologous recombination is higher than when other cells are used (see Table 1).

Citation 3 describes a MAR (5′ MAR) in the vicinity of the chicken lysozyme gene, and a method is described whereby in a system wherein a structural gene such as a CAT gene is made to be expressed by inserting DNA (herebelow called an expression unit) having the aforementioned structural gene such as a CAT gene linked to an enhancer and a promoter into a chromosome, by inserting the aforementioned 5′ MAR into the vicinity of the aforementioned expression unit, the expression of a structural gene incorporated into a chromosome is improved (See Fig. 1, Table 1).

Citation 4 describes a method for controlling the transcription of a gene by using a tetracycline inducible promoter in animal cells such as DT-40 cells.

Citation 5 describes homologous recombination by transducing an EBFP (a variant wherein the 66th amino acid of EGFP is changed to Tyr) gene and a gene for an EGFP derivative (a fusion protein of GFP and EGFP) with a base sequence similar to said EBFP, into animal cells such as DT-40.

Citations 6-7 describes a 3′ enhancer for the chicken immunoglobulin light chain.

Citation 8 describes an enhancer region of MMTV, called GRE (glucocortisoid responsive element), that exists in the vicinity of an MMTV promoter.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/JP03/16496

Supplemental Box Relating to Sequence Listing

Continuation of Box No. I, item 2:

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, this report was established on the basis of:

   a. type of material

   ☒ a sequence listing
   ☐ table(s) related to the sequence listing

   b. format of material

   ☐ on paper
   ☒ in electronic form

   c. time of filing/furnishing

   ☐ contained in the international application as filed
   ☒ filed together with the international application in electronic form
   ☐ furnished subsequently to this Authority for the purposes of search and/or examination
   ☐ received by this Authority as an amendment* on _____

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

* *If item 4 in Box No. I applies, the listing and/or table(s) related thereto, which form part of the basis of the report, may be marked "superseded."*

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of:

Box No. V

The invention recited in Claim 1 does not have novelty and inventive step over Citation 1.
The locations of the genes, base sequences similar to said genes, and transcription promoters for the invention recited in Claim 1 and the invention recited in Citation 1 cannot be differentiated in terms of wording, and for the invention recited in Citation 1, those skilled in the art could readily have placed one gene upstream on the 5′ side of the other gene for which transcription is to be controlled.

The inventions recited in Claims 2, 4-7 do not have an inventive step over Citation 1 and Citation 2.
The DEX inducible MMTV promoter recited in Citation 1 is such that, by the addition of DEX, transcription from the promoter is activated, so it is recognized that the enhancer region of MMTV called GRE is included (see Citation 8).
Further, as the somatic animal cells to be used in the method for inducing homologous recombination recited in Citation 1, those skilled in the art could readily have used, for example, the DT-40 cells described in Citation 2.

The inventions recited in Claims 4-7 do not have an inventive step over Citations 1-2 and Citation 3.
Whereas in the method for inducing homologous recombination described in Citation 1, transcription is activated from a gene incorporated into a chromosome in order to improve the efficiency of homologous recombination, a method whereby, during a similar incorporation of a gene into a chromosome, a chicken 5′ MAR is inserted in the vicinity of an expression unit containing a gene to be incorporated into a chromosome in order to activate the expression of said gene, is publicly known, as described in Citation 3.
Therefore, the insertion of the chicken 5′ MAR described in Citation 3 in the vicinity of an expression unit containing a gene and a DEX inducible MMTV promoter, in a method for inducing homologous recombination using DT-40 based upon Citations 1-2, could readily have been conceived of by those skilled in the art.

The inventions recited in Claims 8, 11 do not have an inventive step over Citations 1-3, Citation 4, and Citations 6-7.
In a method for inducing homologous recombination based upon Citations 1-3, the use of, for example, the tetracycline inducible promoter well-known to those skilled in the art described in Citation 4, and further, for example, the chicken 3′ enhancer well-known to those skilled in the art described in Citations 6-7, in place of the DEX inducible MMTV promoter, in the method for inducing homologous recombination, could readily have been conceived of by those skilled in the art.

The inventions recited in Claims 9-11 do not have an inventive step over Citations 1-4, 6-7, and Citation 5.
In a method for inducing homologous recombination based upon Citations 1-4, 6-7, to carry out the homologous recombination of derivatives of EBFP and EGFP described in Citation 5, and at that time, to use EGFP, which was well-known to those skilled in the art, and ECFP, which is homologous to EGFP, as genes with which to carry out the homologous recombination, could readily have been conceived of by those skilled in the art.